Morphometric Differentiation of *Pratylenchus neglectus* (RENSCH, 1924) and *P. gotohi* n. sp. (Nematoda : Pratylenchidae)

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Six Japanese populations ascribed to *Pratylenchus neglectus* were compared in morphology and with statistics. Variability in outlines of lip region, spear knobs and tail terminus were observed: tail terminal outline is stable within a population and slight difference is recognized between some populations. Five morphometrics were selected to discriminate the 3 groups within the *P. neglectus* complex by the stepwise linear discriminant function analysis. The use of these selected characters were verified by the canonical discriminant function analysis and principal component analysis. The groups were identified as *P. neglectus* and 2 new *Pratylenchus* species, then characterized by the selected characters. *P. gotohi* n. sp., a possible sibling species of the *P. neglectus*, is described. The new species is distinguished from *P. neglectus* by the thicker spear knobs, posterior excretory pore, shorter postvulval uterine branch, lower m-value, larger number of tail annules (20 *vs.* 17 in mean values). *Jpn. J. Nematol.* **21**: 26-42 (1991).

Key words : *Pratylenchus neglectus* complex, *Pratylenchus gotohi* n. sp., taxonomy, statistical differentiation.

Pratylenchus neglectus (RENSCH, 1924) is a well documented nematode pest of the tobacco, cereals and forage, distributing in the temperate regions of the world, hence the nomenclature of this nematode as such has an extensive usage. LOOF[10] was the first to establish the taxonomy of this species by studying mainly the variability of the tail morphology among populations and within populations derived from a single female, concluding *P. minyus* SHER & ALLEN, 1953 as a synonym of this species. Two other species have been synonymized with this species, i.e. *P. capitatus* IVANOVA, 1968 (by LOOF[11]) and *P. neocapitatus* KHAN & SINGH, 1975 (by FREDERICK & TARJAN[4]; RYSS[16]).

Unfortunately, the *P. neglectus* have not yet been fully established on the morphological basis: in fact, except in tail morphology, degrees on variability of the so-called diagnostic characters of this species have not been documented by means of the variability study as done by ROMAN and HIRSCHMANN[15]. It is noteworthy that LOOF[10] recognized the differences in host range between distinct allopatric populations of *P. neglectus* and suggested a race or possibly species complex within this species.

GOTOH[5, 6] reported a species similar to *P. neglectus* from meadow in Japan denoting it as *Pratylenchus* sp. 3. Although GOTOH[6] could not give conclusive evidences to differentiate between his *Pratylenchus* sp. 3 and *P. neglectus*, he found that they were distinct by some differences in the features of spear knobs, lip width and tail terminus. A similar population was also reported by MIZUKUBO, et al.[13] as a possible new species. By unknown reasons, these populations have been found only in uncultivated soils, while the *P. neglectus* found in cultivated soil, and the former will

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probably prove to be biologically discrete sibling species of the latter. Our present work better demonstrates the statistical differentiation between sibling *P. neglectus* complex. The stepwise linear discriminant function analysis was performed to find out the effective characters for discrimination of the groups. The canonical discriminant function analysis and the principal component analysis were used to verify the use of characters.

MATERIALS AND METHODS

SPECIMENS. Six populations of the root lesion nematodes have been studied. Their codes, hosts, localities, year of collection, collector, fixative and mounting media are as follows: 1: Italian rye grass (*Lolium multiflorum* LAM.), Yotsukaido (Chiba Pref.), 1979, N. MINAGAWA, TAF, glycerin by slow method; 2: *Pennisetum alopecuroides* (L.)., Nishigoshi, (Kumamoto Pref.), 1980, N. MINAGAWA, TAF, glycerin by slow method; 3: *Sasa* sp., Nishigoshi, 1980, N. MINAGAWA, TAF, glycerin by slow method; 4) *Imperata cylindrica* (L.) var. *koenigii* (RETZ.), Nishigoshi, 1980, N. MINAGAWA, TAF, glycerin by slow method; 5) mixed vegetation of *Miscanthus sinensis* ANDERSS. and *Sasa* sp., Kokonoe (Oita Pref.), Z. SANO, TAF, glycerin by glycerol-ethanol method; 6) cabbage (*Brassica oleracea* var. *capitata*), Mizobe (Kagoshima Pref.), K. NAKASONO, TAF, lactophenol.

EXAMINATION OF SPECIMENS. The specimens were observed at oil immersion 2,340× for all features by a Olympus BH-2 light microscope equipped with a Normarski interference contrast system. Measurements were made using an eyepiece micrometer.

CHARACTERS CONSIDERED FOR THE STUDY. At first the correlation matrix of 45 morphometrics (including coefficients) in the population combined (n=28) are examined, which is not shown here to save space. Many morphometrics were significantly correlated with $r>|\pm 0.47851|$ ($P<0.05$) and extremely high correlations ($r>|+0.7|$)were found between tail length and TANN ($r=0.78543$); tail length and c ($r=-0.78103$); Tail/V-a and c ($r=-0.76311$); postvulval uterine branch (PUB) and G2 ($r=0.94042$); PUB and U ($r=0.88309$); U and G2 ($r=0.84756$); conus length and M ($r=0.74962$); KBH and ratio spear/lip width ($r=0.76846$); KBH and ratio knob width/height ($r=-0.87951$); median bulb distance and ratio procorpus/spear ($r=0.87950$) along with some very high correlations found between L, VL and L' or those between maximum body width, vulval body width and anal body width. No correlation exists between the KBH and knob width ($r=-0.05078$). Consequently, 12 characters (Table 1) were selected to confirm their possible use in the differentiation of the species.

In order to be included in the discriminant function analyses, outline of spear knob was transformed into numerical characters according to the knob shape codes proposed by MIZUKUBO et al[13]. All the linear morphometrics of soft body portion except for DGO and EXPORE were excluded since the specimens were prepared by the different methods. DGO and EXPORE were used expecting that the different preparation of specimens would not largely affect the distance. Tail end outline would be of particular

Table 1. Morphometric characters used in study.

LIPW	: Lip region diameter in μm
SPEAR	: Spear length in μm
M	: Index m (conus length as percentage of spear)
KBIDX	: Spear knob shape indexed according to MIZUKUBO et al.[13]
KBH	: Spear knob height in μm
DGO	: Distance spear to dorsal esophageal gland orifice in μm
EXPORE	: Position of excretory pore from anterior body end in μm
G2	: Postvulval uterine branch as percentage of body length
V	: Index V (vulva position as percentage of body length)
C	: Ratio c (body length divided by tail length)
CC	: Ratio c' (tail length divided by anal body diameter)
TANN	: Number of tail annules

use but was excluded because of the difficulty in objective coding of this characters.

STATISTICAL ANALYSES. The morphometrical measurements were analyzed with the statistical package Seto/B of Kyoritsu Shuppan Co. LTD. on a NEC PC-9801 series personal computer. Stepwise Linear Discriminant Function Analysis (SLDA), Canonical Discriminant Function Analysis (CDFA) and Principal Component Analysis were performed using Seto/B programs DISC34 and PCA for the analyses and display of the data.

RESULTS

MORPHOLOGICAL OBSERVATION. *Lip region shape* : The shapes of the first lip annule of the populations 1 to 5 are much similar (Fig. 1 A-L). These five populations typically show a notch at the apex due to focusing; antero-lateral margins are straight or slightly concave and sloping distally giving impression of convex lip outline. These populations also have 2 lip annules, though rarely unusual 3 lip annules occur in one side (Fig. 1 H); the first lip annule is narrower than the second one with rare exception (Fig. 1 J). Population 6 does not largely differ from other populations in outline (lateral view: Fig. 1 M-O; dorsal view: Fig. 1 P), though the anterior margin of the first lip annule less convex and showing parabolic slope distally (Fig. 1 N), thus giving impression of rounded or nearly flattened outline, which is similar to that of *P. coffeae*. This population has 2 or 3 lip annules: the 3 lip annules occur frequently and exist even on both sides (Fig. 1 O): when 2 lip annules exist, the second lip annule is thicker than the first lip annule (Fig. 1 N, P).

Spear knob outline : The spear knob outlines differ largely between the populations (Fig. 1 A-P). In the population 1 (n=8), spear knobs are thin at bases and recurved distally; distal edges typically pointed (87.5% in frequency : Fig. 1 A-C) or rarely bluntly pointed (12.5%: Fig. 1 D). These forms can be coded commonly 'indented'. Populations 2 to 5 have variable knobs: they are rather thick at bases ; anterior margins sloping and posterior margins rounded and protruded postero-distally ; the portions where both margins met are pointed (Fig. 1 E, F) (often coded laterally directed). Other types are broadly rounded (Fig. 1 G), flattened anteriorly (Fig. 1 H), indented (Fig. 1 I) and rounded (Fig. 1 J-L). Frequencies of the types in each populations are as follows: in the population 2 (n=3), rounded (33.3%), laterally directed (33.3%), flattened anteriorly (33.3%); in the population 3 (n=6), broadly rounded (16.7%), laterally directed (33.3%), flattened anteriorly (33.3%), indented (16.7%); in the population 4 (n=4), broadly rounded (25%), flattened anteriorly (50%), indented (25%); in the population 5 (n=4), rounded (100%). The population 6 has thin spear knobs similar to those in the population 1, i.e. indented (66.7%: Fig. 1 O, P), and anteriorly flattened type occur (33.3%: Fig. 1 M, N).

Tail tip outline : The tail ends show variations but are characteristic between populations (Fig. 2 A-O). Tail ends in the population 1 (n=8) are smooth and somewhat broad apexes of subhemispherical (SHM : Fig. 2 C, E), rounded dorsally and pointed ventrally (=trapezoid) (TRP: Fig. 2 A, B) or clavate (CLA : Fig. 2 D) in the following frequencies (in parentheses): SHM (50%), TRP (37.5%) and CLA (12.5%). The terminal smooth cuticles of this population are often longer in dorsal than in ventral side. In the populations 2 and 3 tail ends can be categorized into bluntly pointed (BLP: Fig. 2 G, J) or SHM (Fig. 2 F, H, I) but 5 out of 9 were irregularly notched at apexes (Fig. 2 F, G, I, J). Frequencies of the categorized outline are as follows (in parentheses): in the population 2 (n=3), BLP (66.7%), SHM (33.3%); in the population 3 (n=6), BLP (66.7%), SHM (16.7%), truncate (16.7%). In the population 4 (n=4), tail ends show stable outline of smooth and bluntly pointed (Fig. 2 K: 100%). Tail ends of the population 5 are also stable with smooth outline of SHM (100%). In the population 6 (n=4), tail ends are smooth (Fig. 2 L, N, O) or notched (Fig. 2 M), with SHM outline (100%). The trapezoid outline is not observed in the populations 2-6.

STATISTICAL ANALYSES. Twelve selected characters were analyzed by SLDA. The partial *F*

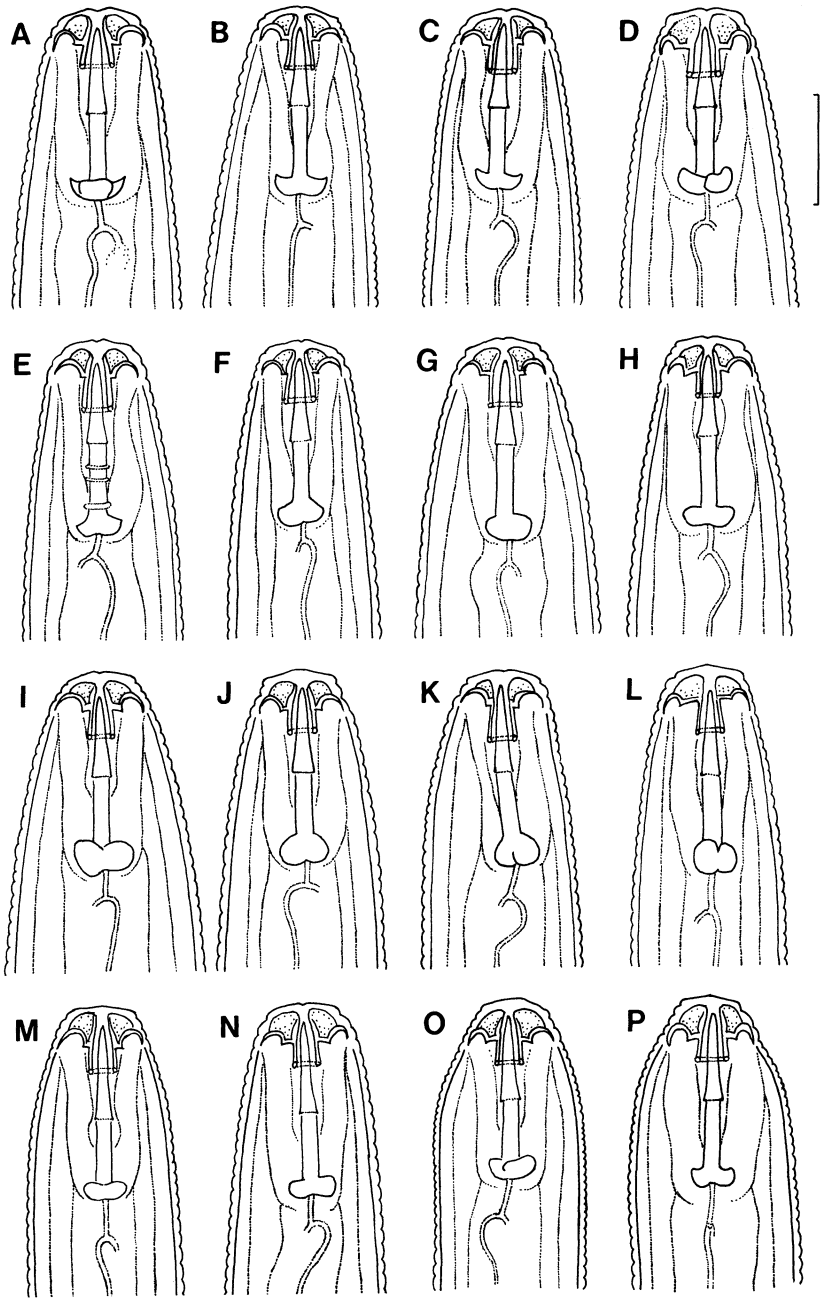

Fig. 1. Anterior body ends of *Pratylenchus neglectus* complex. Female. A-D : Population 1 (from Italian rye grass in Yotsukaido, Chiba); E-H : Population 3 (from *Sasa* sp. in Kuroishi, Kumamoto); I : Population 4 (from *Imperata cylindrica* in Kuroishi, Kumamoto); J : Population 2 (from *Pennisetum alopecuroides* in Kuroishi, Kumamoto); K, L : Population 5 (from *Sasa* sp. + *Miscanthus sinensis* in Kokonoe, Oita); M-P: Population 6 (from cabbage in Mizobe, Kagoshima). Scale bar=10 μm.

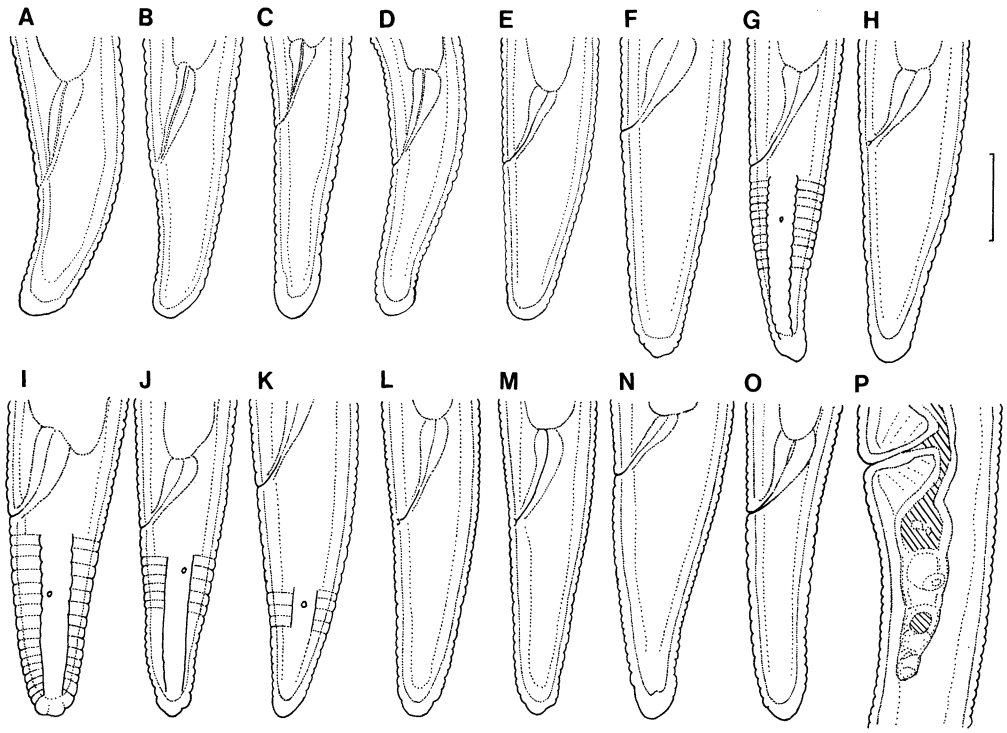

Fig. 2. Tail variation of *Pratylenchus neglectus* complex. Female. A-E : Population 1; F-J : Population 3; K : Population 4; L-O : Population 6. Vulval region ; P : Population 6. Scale bar=10 μm.

statistics for 5 variables (EXPORE, KBH, G2, V and M) in the final model (Table 2) were greater than 3.467 (F^{2}_{21} (0.05) =3.467) and this indicated that only the 5 characters significantly contributed to discrimination of the groups. Other 7 characters (C, CC, SPEAR, TANN, LIPW, KBIDX and DGO) had partial F statistics smaller than 3.467, and did not significantly contribute to the discrimination between the groups. Total difference between the groups diagnosed by lambda statistics of WILKS was as small as 0.0415828 and was significant at 1% level ($F_0=17.1772>F^{10}_{44}$ (0.01) =2.754). Table 3 shows that there are no classification errors between 3 groups by the 5 selected characters. The canonical structures (Table 4) by CDFA and all the specimens plotted on CAN1 (X) and CAN2 (Y) (Fig. 3) were displayed.

Principal Component Analysis was performed to confirm the use of the characters in separation of the groups. This analysis gives objective image of the groups by clustering the homogeneous members when the entity studied is heterogeneous in nature, although distances among species in principal components space can not be interpreted. Table 5 shows eigenvalues and eigenvectors of the correlation matrix of the 5 selected characters. Scatter diagram, which result from plotting the specimens after ordination, shows 3 separable clusters in Fig. 4 (plotted on Principal Component (PC) 1 (X) and PC 2 (Y)). The clusters are identical to the 3 groups classified by the SLDA.

DISCUSSION

GOTOH[6] distinguished his *Pratylenchus* sp. 3 from *P. neglectus* by the differences in the spear

Table 2. Discriminant functions in the 3 groups of *Pratylenchus neglectus* complex.

Variables	:	Partial F	1	2+3+4+5	6
EXPORE	:	11,4120	−1.8785	−0.3037	−1.3553
KBH	:	9.5354	−6.7991	9.0479	0.4468
G2	:	9.0419	3.8734	10.0274	8.7628
V	:	7.2356	68.4207	64.8430	65.3127
M	:	3.6246	28.4204	27.4967	29.2617
(Variables not in the functions)					
LIPW	:	2.1892	—	—	—
KBIDX	:	2.1749	—	—	—
DGO	:	1.0470	—	—	—
TANN	:	1.0113	—	—	—
SPEAR	:	0.7127	—	—	—
CC	:	0.6837	—	—	—
C	:	0.1699	—	—	—

Note : Partial F d.f.=(2, 21); Lambda=0.0415828; F=17.1772 d.f.=(10, 44)

Table 3. Result of discrimination between 3 groups of *Pratylenchus neglectus* complex.

Specimens (code)	Number of case	Number of correct	Percent correct	Number of cases classified 1	2+3+4+5	6
1	8	8	100.0	8	0	0
2+3+4+5	17	17	100.0	0	17	0
6	4	4	100.0	0	0	4

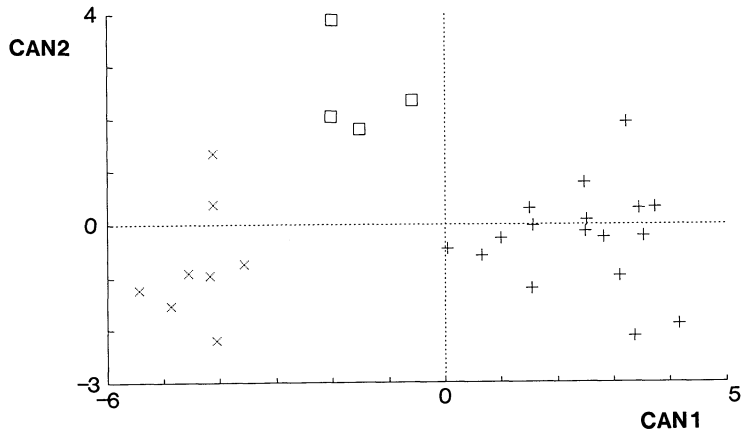

Fig. 3. *Pratylenchus neglectus* complex. Comparison of 3 groups using 5 selected variables. Symbols represent groups (populations): ×=Gl (1), +=G2 (2+3+4+5), □=G3 (6). CAN1, CAN2 : Canonical discriminant functions 1 and 2.

knob shape, tail shape and lip width. However, since the high variability of spear knob outline have been documented in several *Pratylenchus* species, the knob shapes usually have not been considered diagnostic character. Again, LOOF[10] described narrowly rounded tail as a variation of *P. neglectus*. Hence, these would not be regarded as complete proof to claim the distinction of his specimens from *P. neglectus*. Lip region diameter has seldom been measured and is unknown how far they vary.

Table 4. Canonical variates in the 3 groups of *Pratylenchus*.

Variates	1	2
EXPORE	0.2353	−0.0444
V	−0.4895	−0.5299
KBH	2.3212	0.2040
G2	0.8528	0.7615
M	−0.1649	0.4035
constant	21.1116	22.1476

However, the statistical analysis selected the following 5 characters to be successful in discrimination of the *P. neglectus*, *Pratylenchus* sp. 3 *sensu* GOTOH, 1970 and a possible new species. We would discuss the properties of these and others below.

EXPORE : This is the most contributed character to the discrimination of the 3 groups so far as the populations examined are concerned. Note that the characters on distance of soft body

Table 5. Eigenvalues and eigenvectors of the correlation matrix.

Variables	1	2	3	4	5
KBH	0.49243	−0.14429	−0.59888	0.54247	−0.28943
EXPORE	0.53075	0.23972	−0.22270	−0.27303	0.73260
V	−0.05185	0.88370	0.15866	0.43534	−0.04112
G2	0.43616	−0.29946	0.72668	0.40967	0.15558
M	−0.53187	−0.22609	−0.19626	0.52329	0.59468
Eigenvalues	2.42451	1.15652	0.68633	0.38231	0.35033
Proportion	0.48490	0.23130	0.13727	0.07646	0.07006
Cumulated proportion	0.48490	0.71621	0.85347	0.92994	1.00000

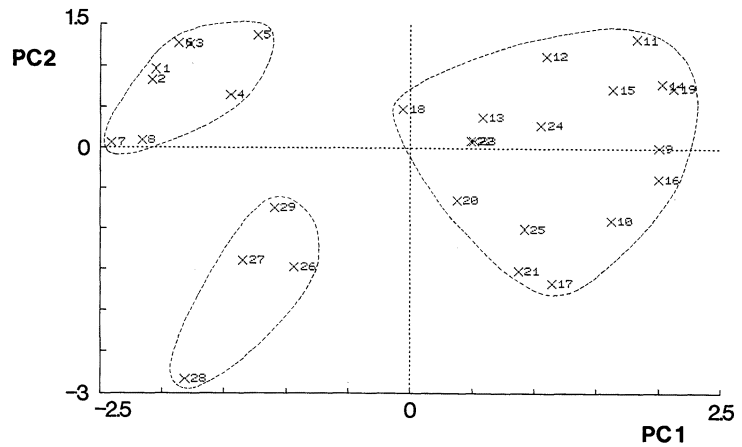

Fig. 4. *Pratylenchus neglectus* complex. Scatter diagram of specimens grouped according to the ordination after principal component analysis. Numbers represent specimens : 1−8= G1, 9-25=G2, 26−28=G3. PC1, PC2 : Principal components 1 and 2.

portion of nematode such as body length (L), body width vary according to preparing methods of the specimens. The EXPORE may be one of these characters and our specimens were prepared by the different methods. Nevertheless, the use of this character for the statistic analysis would be justified by the following 3 considerations: i) EXPORE is more informative than standardized index E.P. (EXPORE as percentage of body length). This index has often been used in Japan, but is found less usable as the coefficient of variability (CV.) of the E.P. is generally greater than that of EXPORE. This is further verified by the indefinite correlation ($r=0.57473$) between L and EXPORE, which is statistically significant but not as high as those recognized between L and VL with correlation being almost 1.0. ii) Many researchers reported that morphometrics of the nematode differed between methods by which the specimens were prepared. BROWN and TOPHAM[1] showed using *Xiphinema* that TAF fixative did not cause shrinkage in the esophageal region. Moreover, when the TAF-fixed specimens were transferred to glycerin, they observed, the difference of glycerol-ethanol method and slow method did not affect the esophagus length. Although it has not been proven if the same is true for *Pratylenchus* species, we consider it possible that the EXPORE is usable in the analysis of *Pratylenchus* so far as the same fixative is used throughout the investigation. iii) The third group was mounted in lactophenol: the body length and EXPORE of the specimens mounted in lactophenol (n=10) is ca. 8% longer comparing with those processed by glycerol-ethanol method (n=9) according to the senior author's data taken from *Filenchus* species. Nevertheless the EXPORE of the third group is significantly shorter than that of the second group : use of EXPORE of the third group will be justified so far as the present comparison is concerned.

GOTOH & OHSHIMA[7] and YAMADA[20] independently calculated 16.8% and 17.9% E.P. (given in mean values) to lactophenol-mounted *P. neglectus*. From these values, we recalculated each as 76 μm and 70 μm EXPORE (in mean values), which were corrected to 70 μm and 65 μm EXPORE of glycerol-ethanol processed specimens multiplied by 0.926 (100/108). These values do not largely differ from the value of the first group. It should be further noted that the EXPORE reported for *P. neglectus* is variable depending on the authors as 66-78 μm (after CORBETT[2]), 59.6-82.2 μm (after VAN DEN BERG in TOWNSHEND & ANDERSON[19]), 81 (75-87) μm (after TOWNSHEND & ANDERSON[19]) and 84 μm (after ZEIDAN & GERAERT[21]). Parts of the differences may be interpreted as the effect of the preparation methods, but it is possible to consider some differences are genetic between these populations.

KBH : This character was highly correlated to knob width/height ratio (KBWH), so that the latter was discarded. KBH, knob width (KBW) and KBWH were compared by GOTOH[6] between *P. neglectus* and his *Pratylenchus* sp. 3. He reported 4.5 μm (4.3-4.7) wide, 2.0 μm (1.9-2.1) high and 2.3 (2.2-2.4) KBWH for the spear knobs of *P. neglectus* (n=20) vs. 4.1 μm (4.0-4.2), 2.4 μm (2.2-2.6) and 1.7-1.9 (1.8), respectively for his *Pratylenchus* sp. 3 (n=20). Differences were obvious and he considered his species distinct. Our measurements on the spear knob height in the first group (*P. neglectus*) and the second group (*Pratylenchus* sp. 3, Table 6) are close to the above, hence, GOTOH's comparison would be confirmed. Two specimens reported from Sudan as *P. neglectus* have, however, obviously higher spear knobs[21]. The KBH is also important with respect to its influence on the outline of spear knobs as discussed in the section of KBIDX.

G2 : This character is much effective so far as the populations examined are concerned, though further study of variablility is required. The use of G2 or direct length of postvulval uterine branch (PUB) is advocated rather than ratio U (PUB length divided by vulval body diameter), which less contribute to the discrimination according to the preliminary study. Note that ratio U is affected by the body width, which is variable by the effects of environmental factors, maturity and artificial factors such as preparation of the specimens.

GOTOH & OHSHIMA[7] and YAMADA[20] calculated the very similar G2 values for *P. neglectus*: i.e. 3.8% (2.8-5.4) (n=29) and 3.7±0.3% (2.8-4.7) (n=15), respectively, which again consistent to the

Table 6. Measurements of the six samples of *Pratylenchus neglectus* complex.

Locality	Yotsukaido	Nishigoshi			Kokonoe	Mizobe
Sample codes	1	2	3	4	5	6
n	8	3	6	4	4	6
L (μm)	386 : 26.4*	437 : 13.2	432 : 38.9	412 : 19.8	412 : 26.7	407 : 22.3
a	23.8 : 2.78	23.8 : 1.29	21.0 : 3.65	19.8 : 0.78	26.0 : 0.96	24.0 : 1.39
b	6.2 : 0.79	6.1 : 0.71	5.4 : 0.74	5.0 : 0.30	5.1 : 0.47	5.4 : 0.30
b'	4.4 : 0.51	3.9 : 0.41	3.9 : 0.46	3.6 : 0.19	3.3 : 0.23	3.6 : 0.12
c	20.3 : 2.60	18.7 : 2.45	17.2 : 1.93	17.2 : 1.03	18.7 : 1.40	16.6 : 1.25
c'	2.0 : 0.34	2.2 : 0.17	2.2 : 0.14	2.2 : 0.18	2.5 : 0.20	2.5 : 0.34
V (%)	82.2 : 0.54	81.0 : 1.66	81.5 : 1.34	80.1 : 1.41	80.9 : 1.20	79.5 : 1.37
G2 (%)	3.7 : 0.34	5.8 : 1.30	6.0 : 1.05	4.8 : 0.20	4.6 : 0.10	5.4 : 0.44
Spear (μm)	15.2 : 0.44	16.1 : 0.38	15.5 : 0.47	15.7 : 0.56	16.0 : 0.48	15.6 : 0.32
m (%)	50.7 : 2.09	46.9 : 0.21	47.3 : 1.25	47.9 : 1.62	49.1 : 1.13	51.7 : 1.06
DGO (μm)	2.8 : 0.34	2.0 : 0.08	2.1 : 0.49	2.3 : 0.28	2.9 : 0.17	3.0 : 0.34
Esophagus (μm)	63 : 7.4	72 : 7.1	76 : 5.0	84 : 8.7	81 : 4.5	73 : 6.9
EXPORE (μm)	67 : 3.5	80 : 3.6	76 : 3.0	79 : 4.1	75 : 2.4	65 : 3.8
E.P. (%)	17.5 : 1.30	18.3 : 1.17	18.6 : 2.20	19.1 : 0.52	18.3 : 0.62	16.0 : 0.57
V-a (μm)	50 : 4.1	59 : 3.1	52 : 5.7	58 : 3.2	57 : 6.3	60 : 5.6
Tail (μm)	19 : 2.7	24 : 2.8	24 : 2.0	24 : 2.1	22 : 2.9	25 : 2.5
PUB (μm)	14 : 1.5	25 : 6.0	25 : 5.8	20 : 0.9	19 : 1.1	22 : 2.1
U	1.0 : 0.08	1.6 : 0.24	1.4 : 0.27	1.1 : 0.11	1.3 : 0.08	1.4 : 0.09
Tail annules.	17 : 1.4	19 : 1.7	20.5 : 1.2	20 : 1.0	20 : 3.6	21 : 2.9
Knob width	4.5 : 0.6	4.6 : 0.33	4.3 : 0.52	4.7 : 0.34	4.2 : 0.57	4.2 : 0.14
KBH (μm)	1.8 : 0.14	2.6 : 0.35	2.6 : 0.44	2.3 : 0.33	2.9 : 0.20	2.1 : 0.24
Knob w/h	2.6 : 0.39	1.8 : 0.30	1.7 : 0.19	2.0 : 0.39	1.4 : 0.32	2.0 : 0.30
Lip height	2.4 : 0.17	2.0 : 0.06	2.2 : 0.28	2.2 : 0.16	2.4 : 0.25	2.4 : 0.18
LIPW (μm)	8.1 : 0.38	7.6 : 0.06	7.7 : 0.23	7.6 : 0.22	7.4 : 0.15	7.9 : 0.36

*mean : S.D.

measurements of the first group. GOTOH[5, 6] reported for his *Pratylenchus* sp. 3 only on the range of G2 as being 3-6%, which overlaps both the G2s of the first and the second groups. This inconsistency means: 1) G2 is actually more variable characters in *Pratylenchus* sp. 3 or 2) GOTOH's description was insufficient. Further study of variability of this character is desirable.

V : The V character among 3 groups looks almost the same with some overlapping variability. However, use of this character is justified by the present statistical study of the characters. Probably, the slight difference in the average of this value is usable to recognize species. Reversely, there reported different mean values for index V of *P. neglectus* : 83.3% (after LOOF[10] from paratypes of *P. minyus* SHER & ALLEN[18]), 81.8%, 80.9%, 81.6%, 81.8%, 81.3%, 82.1% (all after LOOF[10]), 82% (after KHAN & SINGH[9] for *P. neocapitatus* (syn. of *P. neglectus*)), 82%, (after TOWNSHEND & ANDERSON[19]), 82.5% (after GOTOH & OHSHIMA[7]), 82.9% (after YAMADA[20]), 83% (after RYSS[16]). IVANOVA[8] also reported the V-value of 80-83.5% which suggests the mode of ca. 82% for the *P. capitatus* (syn. of *P. neglectus*). The large deviation (83-85%, n=2) appeared in Sudanese population[21]. Some of these differences may or may not reflect the genetic difference. We would like to point out that 82% is the mode of these mean values from different geographic samples. GOTOH[5, 6] reported for his *Pratylenchus* sp. 3 the 81% (79-83) V-value which agree with our measurements in the second group.

M : The m-value generally has variability as found in the first group. However, populations assigned to the second group are unique because none of the member has m-value exceeding 50%. This make it possible for index 'm' to contribute significantly to discrimination of the groups.

The third group also has m-value always exceeding 50%, though true variability of the character in this group is unknown.

The following characters are not considered contributing at 5% level to the discrimination. However, some of them would prove to be useful for the discrimination when the sample size comes larger, which would rise the partial F statistics of these characters.

LIPW : Although this character does not significantly contribute to the separation of the groups, some differences exist between the first group (7.5-8.4 μm wide) and the other 2 groups (7.1-7.9 μm wide). GOTOH[6] compared this character between *P. neglectus* (8.4 μm (8.2-8.6): n=20) and his *Pratylenchus* sp. 3 (7.4 μm (7.3-7.6): n=20). This morphometrics given for *Pratylenchus* sp. 3 agrees with our measurements in the second group, while measurements from *P. neglectus* slightly deviate from those for the first group (*P. neglectus*). The LIPW would be variable among the *P. neglectus* populations and further study of the variability in this species is required. ZEIDAN & GERAERT[21] described 9.0-9.5 μm wide lip region for *P. neglectus* from Sudan.

KBIDX : At the first glance there are some differences in the knob outline between the first and the second groups. TOWNSHEND & ANDERSON[19] described the knobs of *P. neglectus* as "typically indented on anterior margin". The same type is found in the first group with less variation (Fig. 1 A-D). The second group conversely has considerable variations in this character even within a population (Fig. 1 E-L). The third group (Fig. 1 M-P) shows both flattened and indented knobs. We notice, however, that impressions of knob shape are to some extent affected by the height of the knob. We may distinguish thick indented knobs (Fig. 1 I) from thin indented knobs (Fig. 1 A-D), which can be commonly categorized as "indented". Eventually there are overlapping variability in the categorized knob shape between the first and the second groups. GOTOH[6] described the knobs of his *Pratylenchus* sp. 3 as "stylet knobs are rounded ; though they are slightly laterally dilated in some individuals, their width/height ratio are smaller than those obtained in *P. neglectus*" (translated from Japanese). His recognition of knobs as being rounded may be derived from the impression resulted from the thickness (height) of knobs and may not directly mean the rounded shape, though the latter may actually occur as a variation. It is noteworthy that ZEIDAN & GERAERT[21] reported high rounded knobs for *P. neglectus* from Sudan.

DGO : TOWNSHEND & ANDERSON[19] described for *P. neglectus* the 3.0-4.8 μm distant DGO. While, YAMADA[20] measured narrower, 2.9±0.2 μm (2.4-3.8) distant DGO for a *P. neglectus* population in Japan, which is identical to our measurements of the first group. GOTOH[5, 6] described for his *Pratylenchus* sp. 3 the 2.5-3.0 μm DGO, which is slightly longer than our dimension for the second group (Table 6). It should be noted that 2.0-2.5 μm DGO was described for *P. neglectus* from Sudan[21], which is shorter than those of Canadian population and even of Japanese population (the first group).

TANN : This is one of the 12 diagnostic characters selected by LOOF[11] for *Pratylenchus* taxonomy. SEINHORST[17] was the first to take the number of tail annules into taxonomic considerations, describing 17 (16-19) annules for *P. neglectus*. TOWNSHEND & ANDERSON[19] took the states of this character as diagnostic for *P. neglectus*, which "usually with 18 (15-20) annules". CORBETT & CLARK[3] reported 16 (11-19) tail annules for the latter species. Drawings of 31 tails of *P. neglectus* by LOOF[10] show 17.5 (14-22) annules and their frequencies (in parentheses) are 14 (9.7%), 15 (6.5%), 16 (19.4%), 17 (12.9%), 18 (22.6%), 19 (12.9%), 20 (6.5%), 21 (6.5%), 22 (3.2%). This pattern is similar to the first group (n=11), where frequencies of annules are 14 (9.1%), 15 (9.1%), 16 (27.3%), 17 (18.2%), 18 (18.2%), 19 (18.2%). While in the second group (n=17), frequencies of annules are 17 (11.8%), 18 (11.8%), 19 (5.9%), 20 (17.6%), 21 (47.1%), 25 (5.9%). In the third group (n=7), they are 18 (42.9%), 22 (14.3%), 23 (14.3%), 24 (28.6%). It can be concluded that *P. neglectus* has the 16-18 tail annules as the mode of range. Hence, we consider the pattern of the distribution is diagnostic in our materials when large number of specimens are examined. Although, because of the overlapping variability, this character shows

less contribution to the separation of the groups on the statistical basis, this is usable to confirm the identity of the species and in some case even diagnostic. CORBETT & CLARK[3] recognized "intraspecific variability in this character with many species having between 13 and 24 annules on the ventral surface of the tail" and concluded that "it is not a useful character in differentiating species", though they noted one or two species differed from the norm in the number of annules on the tail. It should be noted IVANOVA[8] illustrated 16 and 20 annules for *P. capitatus*; KHAN & SINGH[9] illustrated 16 and 19 for the *P. neocapitatus*, which fall into tail annule range for *P. neglectus*. Conversely, 20 and 24 tail annules illustrated for Sudanese *P. neglectus* may be unusually larger for this species.

Finally it should be stressed that comparative study using more than 2 real *P. neglectus* populations with large sample size is indispensable for further objective separation. A SEM observation of the first lip annule may provide these species with some additional taxonomic information.

CONCLUSION

Based on the statistical analysis, Japanese populations of *P. neglectus* were classified into 3 groups, which represent independent species.

1) *P. neglectus* : A population from Italian rye grass in Yotsukaido, Chiba can be identified as real *P. neglectus*. This species is characterized by the 2 lip annules, the first lip annule with convex anterior margin, vulva at 82%, smooth tail terminus. *P. neglectus* can be distinguished from closely resembled species by the shorter distance of excretory pore (63-74 μm; TAF, glycerin), short post vulval uterine branch (PUB) (G2=3.7-3.8% (in mean values)), posterior location of vulva (V=82% in mean value), lower spear knobs (1.8-2.0 μm (in mean value)) and 51% m value (mean). The following characters are of particular use to confirm the identification: much flattened and indented spear knobs, trapezoid tail outline with dorsal terminal smooth region often longer than ventral one and fewer number of tail annules (17 (15-20)). It is noteworthy that the Canadian populations described and illustrated by TOWNSHEND & ANDERSON[19] differ from the Japanese population in the lip region outline of being distinctly offset and the first and the second lip annules of about equal width. Japanese populations have lips continuous to (or very slightly offset from) body contour and their first lip annule fairly narrower than the second lip annule. These character-states in Japanese populations rather agree with those given for *P. minyus* by SHER & ALLEN[18] and *P. neglectus* by LOOF[11].

2) *Pratylenchus* sp. 3 *sensu* GOTOH, 1970 : Populations from Nishigoshi and Kokonoe show some variability in some morphology but have distinctive characters identical to *Pratylenchus* sp. 3 of GOTOH[5, 6]. These populations are described as a new species in the following systematics section, in which the character-states differentiate this species from related ones are given. Close similarity in the states of many diagnostic characters to *P. neglectus* may suggest allocation of this species to subspecific rank of the latter. Such allocation is, however, not reasonable because of the sympatric distribution of the latter, hence, we would like to regard the species as a sibling species of *P. neglectus*.

3) *Pratylenchus* sp. : A population from cabbage in Mizobe, Kagoshima is a possible new species. This species comes close to *P. neglectus* , *P. hexincisus* TAYLOR & JENKINS, 1957 ; *P. alleni* FERRIS, 1961 ; *P. jordanensis* HASHIM, 1983 ; *P. scribneri* STEINER, 1943 and *Pratylenchus* sp. 3 *sensu* GOTOH, 1970[5] by the following character-states : 2 lip annules, spear at most 18 μm, smooth tail ends, posterior vulva (V$\geqq$77% in mean) and shorter body (L<500 μm in mean). i) This species differs from *P. neglectus* (=PN) by having 2 or 3 lip annules on either or on both sides (occurrence of 3 lip annules is extremely rare in PN), anterior vulva (V=79.5% *vs.* 82% in mean), longer PUB (G2=5.4% *vs.* 3.7% in mean). Further differences are in lower c-value (17 *vs.* 20 in mean), larger number of tail annules (21 *vs.* 17 in mean), different lip outline (rounded *vs.*

convex) and different tail outline (trapezoid outline of PN did not found in this species). Further, PN is a parthenogenetic species while this species has males and with rounded spermatheca filled with spermatozoa. ii) From *P. hexincisus*, this species differs by the lateral fields incisures (4 *vs.* 6), slightly posterior vulva (V=79.5% *vs.* 78.0% in mean values), longer PUB (U=1.4 (1.3-1.6) *vs.* ca. 1). Further difference may exist in longer DGO (3.0 μm (2.6-3.4) *vs.* 2.7 μm (1.7-2.5)), smaller c-value (16.6 (14.9-18.6) *vs.* 18.6 (16.1-22.7)) and reproduction mode (bisexual *vs.* parthenogenetic). iii) *P. alleni* comes most close to the present species in many respects. However, this species separated from the *P. alleni* by the longer spear (15.6 μm (15.2-15.8) *vs.* 14 μm (13.5-15)) and frequent occurrence of 3 lip annules. Difference in tail annules (21 (18-24) *vs.* 15-19) is probably important for differentiation of these species. iv) From *P. jordanensis*, this species can be separated by the posterior vulva (V=79.5% *vs.* 77.8% in mean values), frequent occurrence of 3 lip annules, tail outline (smooth *vs.* notched at middle), a-value (24 (22-26) *vs.* 29 (26-32)). Difference are also exist in reproduction mode (bisexual *vs.* parthenogenetic). v) From *P. scribneri* (cf. ROMAN & HIRSCHMANN[15]), present species differs exclusively by the posterior vulva (V=79.5% *vs.* 77.4% in mean values). Other differences are in the m-value (51.7% *vs.* 47.6% in mean values), spear knob height (2.1 μm (1.7-2.3) *vs.* 2.42 μm (1.8-3.0)) shape of spear knobs (flattened or indented *vs.* broadly rounded), DGO (3.0 μm (2.6-3.4) *vs.* 2.2 μm (1.8-2.4)) and reproduction mode (bisexual *vs.* parthenogenetic). There is a remarkable difference EXPORE (65 μm (60-69) *vs.* 88 μm (79-96)), which will not be justified due to different preparation of the specimens. vi) From *Pratylenchus* sp. 3 *sensu* GOTOH, 1970[5], this species can be separated by the anterior vulva (V=79.5% *vs.* 81.0% in mean values), larger m-value (51.7% (50.0-53.0) *vs.* 47.8% (45.8-50.0)) and variable lip annules (2 or 3 *vs.* 2). Additional differences exist in the lip outline (rounded *vs.* convex), spear knob height (2.1 μm (1.7-2.3) *vs.* 2.6 m (2.0-3.3)) and reproduction mode (bisexual *vs.* parthenogenetic).

Above comparisons suggest that present species from Mizobe may be a new species. It should be noted further that this species comes most close to a *Pratylenchus* sp. described from Hokkaido[12] by the consistency of some important characters, i.e. number of lip annule, V-value, spear length, shape of spermatheca and mode in tail annule distribution. However, comparing with present species, the latter has higher knobs (2.5 μm in mean), lower m-value (49.6% in mean value), longer EXPORE (75 μm in mean value), shorter G2 (4.1% in mean value) without differentiation and larger c-value (18.8 in mean value) along with climatic difference in habitat (there would be more than 15°C difference in annual mean temperature between these habitats). Description of the present species will be made elsewhere after the additional specimens are obtained.

4) Sudanese population attributed to *P. neglectus* on the only 2 females bases[21] differs from the latter in many respects of character-states so far reported. Rather some character-states (e.g. lip outline, massive spear knobs, posterior vulva) suggest its relationship to *P. brachyurus*, though the latter has much longer spear of ca. 19 μm. We would like to call attention that occasional *P. brachyurus* has 17 μm long spear. The status of Sudanese specimens is difficult to determine, but we would like to regard the Sudanese population as doubtful *P. neglectus*.

Pratylenchus gotohi n. sp.

Syn. *Pratylenchus* sp. 3 *sensu* GOTOH, 1970

(Fig. 1 E-L; Fig. 2 F-K; Fig. 5)

MEASUREMENTS. *Holotype female (from Kokonoe population)*: L=418 μm; a=27; b=5.1; b'=3.3; c=19.8; c'=2.3; V=81.2%; V'=85.5%; spear=15.8 μm; G1=30.3%; G2=4.6%; U (postvulval uterine branch length divided by vulval body width)=1.4; Tail/V-a=36.8%.

Holotype and paratype females (n=17): L=377-471 μm (422±27.8); a=16.5-27.0 (22.4±3.27); b=4.5-6.6 (5.4±0.68); b'=3.3-4.5 (3.7±0.47); c=14.6-21.4 (17.8±1.76); c'=2.0-2.8 (2.3±0.20); V=

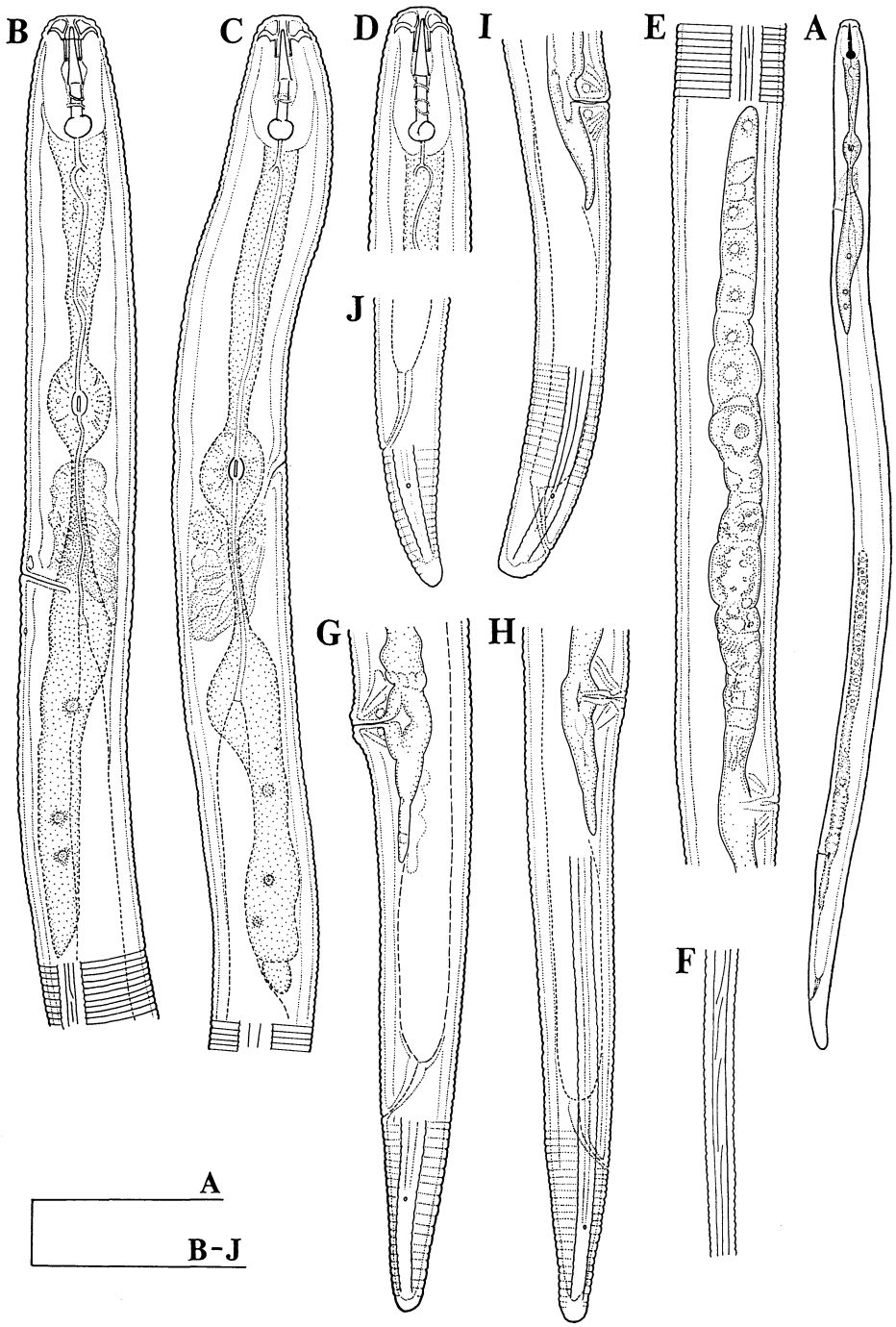

Fig. 5. *Pratylenchus gotohi* n. sp. Female. A : Entire ; B : Esophageal region (normal); C : Esophageal region (abnormal); D : Head ; E : Gonad ; F : Lateral field ; G, H : Posterior body region (normal); I : Posterior body region (abnormal); J : Tail. Scale bars : A=100 μm; B−J=30 μm.

78.3-82.8% (80.9±1.35); V'=83.3-88.2% (85.8±1.32); spear=14.9-16.6 μm (15.8±0.49); m=45.8-50.0% (47.8±1.39); DGO=1.3-3.0 μm (2.3+0.46); MB=61.2-73.8% (66.6±3.52);G2=4.5-8.1% (5.4%±0.99); U=1.0-1.9 (1.4±0.26); Tail/V-a=33-55% (43±5.9); excretory pore/body length=16.8-22.7% (18.6±1.38); knob width/height=1.3-2.5 (1.7±0.32); lip height=1.8-2.6 μm (2.2±0.25); lip width=7.3-7.9 μm (7.6±0.23); knob height=2.0-3.3 μm (2.6±0.40); knob width=3.6-5.0 μm (4.4±0.47); esophagus=67-92 μm (78±7.1); excretory pore=72-85 μm (77±3.5); vulva-anus=44-65 μm (56±5.4); postvulval uterine branch=18-36 μm (22±4.9); tail=20-27 μm (24±2.3); tail annules=17-25 (20.1±1.93); annule width at mid-body=1.2-1.4 μm (n=4).

DESCRIPTION. *Female* (n=17): Body rather short, almost straight to slightly bent when heat relaxed, narrowing fairly just posterior to vulva. Annules fine and rounded. Lip region low (2.2 μm) and relatively narrow (7.6 μm); anterior margins slightly notched at apexes, the lateral edges of the margins straight or slightly concave and sloping distally, giving impression of convex lip outline; with 2 broad annules of equal thickness wihch continuous to or slightly offset from body contour (variation occur in number of lip annules : one out of 17 specimens has dorsal 3 lip annules). Labial framework arched, extending 1-2 annule down into body. Cephalids not seen. Spear with stout impression; conus shorter than a half of entire spear (48%). Basal knobs of spear relatively high (2.6 μm); variable in outline with laterally directed, anteriorly flattened, broadly rounded, indented and rounded variations exist. Dorsal esophagus gland orifice close to spear base (2.3 μm). Metacorpus oval, set off ; valve conspicuous 46-59 μm (52±3.2) from anterior body end. Esophageal gland 101-135 μm (114±10.22) long from anterior body end, ventrally overlapping the intestine, 20-50 μm (35±8.6) long; esophagus gland nuclei line up. Excretory pore level with esophago-intestinal junction (excretory pore in one abnormal female, which is excluded in all measurements, located at metacorpus level). Hemizonid flat, 2-4.5 annules long, same level or immediately anterior to excretory pore ; hemizonion lenticular, 6-14 annules posterior to excretory pore.

Gonad outstretched, oocytes in a row, rarely doubled in anterior portion; spermatheca empty, rounded. Postvulval uterine branch longer than vulval body diameter, with or without rudimentary ovary. Vagina perpendicular to body axis, shorter than vulval body diameter (39-48%).

Tail nearly conoid, evenly tapers to smooth and narrowly rounded (subhemispherical or bluntly pointed) terminus, with no extensive variations but some are irregularly incised ; one abnormal female (Fig. 5 I ; excluded in all measurements) had phasmids shifted anterior to anus. Tail terminal cuticle 2.5-3.0 μm (2.8±0.2) thick ; phasmids centered in lateral fields variable in level on tail.

Lateral fields 4.0-5.0 μm wide or 25-30% of widest body diameter, consist of 3 bands ; inner bands incised by an often broken line; outer margins appear weakly crenate in transmitted light. Lateral fields may extend to tail tip and continue around tail terminus.

Males: Unknown.

TYPE MATERIALS. Holotype female (slide T33-1), 16 paratype females and 2 paratype fourth stage juveniles (slides T33-2—T33-15) deposited in the Herbarium and Insect Museum of the National Institute of Agro-Environmental Sciences (NIAES), Tsukuba City, Ibaraki, Japan.

TYPE HOSTS AND LOCALITY. *Miscanthus sinensis* and *Sasa* sp. (Teradoko, Kokonoe, Oita, 12. viii. 1988, leg. Zen'ich SANO); *Pennisetum alopecuroides* (Kuroishi, Nishigoshi, Kumamoto, 1980, leg. Nozomu MINAGAWA); *Sasa* sp. (Kuroishi, Nishigoshi, Kumamoto, 1980, leg. Nozomu MINAGAWA); *Imperata cylindrica* (Kuroishi, Nishigoshi, Kumamoto, 1980, leg. Nozomu MINAGAWA).

DIAGNOSIS AND RELATIONSHIPS. *Pratylenchus gotohi* n. sp. is characterized by the 2 lip annules, the first lip annule with convex anterior margin, vulva at 81%, longer postvulval uterine branch (PUB) of about 5% of body length, and smooth and rarely incised tail terminus. The narrow lip (7.6 μm), high spear knobs (2.6 μm), subhemispherical or bluntly pointed tail outline with dorsal and ventral terminal smooth region of equal length, number of tail annules (20 (17-25)) are

characteristic for the species. By having 2 lip annules, spear at most 18 μm long, smooth tail, posterior vulva (V≧77%) and short body (L<500 μm in mean value), the new species is separated from all the congeneric species except *P. neglectus* RENSCH, 1924; *P. hexincisus* TAYLOR & JENKINS, 1957; *P. alleni* FERRIS, 1961; *P. jordanensis* HASHIM, 1983, *P. scribneri* STEINER, 1957. i) This species can be distinguished from closely resembled *P. neglectus* by the longer excretory pore (77 μm (72-85) *vs.* 67 μm (63-74) by TAF, glycerin), longer PUB (G2=5.4 μm (4.5-8.1) *vs.* 3.7 μm (3.3-4.3) by TAF, glycerin), anterior vulva (V=81.0% (78.3-82.8) *vs.* 82.2% (81.2-82.8)), higher spear knobs (2.0-3.3 μm vs. 1.7-2.0 μm), lower m-value (47.8% (45.8-50.0) *vs.* 50.7% (47.8-54.2)). Some differences in body habitus (nearly straight *vs.* arched), tail annules (17 *vs.* 20 in mean values), tail terminal outline and spear knob variation range are helpful to separate the 2 species. ii) From *P. hexincisus*, the new species distinguished by the number of lateral fields incisures (4-5 *vs.* 6), posterior vulva (V=81% *vs.* 78% in mean values), longer PUB (U=1.4 *vs.* ca. 1.0 (G2=3.4)). Further, some differences may exist in the height of lip, length of excretory pore between these species. iii) From *P. alleni*, the new species differs by the longer spear (15.8 μm *vs.* 14 μm in mean values). There are additional differences in tail annule (20 (17-25) *vs.* 15-19) and in reproduction mode: *P. alleni* is a bisexual species having rounded spermatheca filled with spermatozoa. iv) From *P. jordanensis*, the new species separated by the posterior vulva (V=80.9% *vs.* 77.8% in mean), longer spear (15.8 μm (15-17) *vs.* 15 μm (14.5-15.0)), relatively short PUB (U=1.4 *vs.* ca. 1) and smaller a-value (17-27 *vs.* 26-32). v) From *P. scribneri* (cf. ROMAN & HIRSCHMANN[15]), the new species exclusively distinguished by the posterior vulva (V=81% *vs.* 77% in mean values). There is difference in excretory pore distance (77 μm (72-85) *vs.* 88 μm (79-96)). However, since ROMAN & HIRSCHMANN[15] mounted the specimens in 2% formalin, direct comparison of their linear morphometrics with our measurements obtained from TAF fixed and glycerin mounted specimens would not be justified.

REMARKS. The scientific name of this species is designated after Dr. Akira GOTOH in commemoration of his contribution to the survey and identification of the Japanese *Pratylenchus* species.

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和文摘要

ムギネグサレセンチュウと *Pratylenchus gotohi* (新種)の計測値による判別

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ムギネグサレセンチュウ関連6個体群（千葉県四街道市の1個体群、熊本県西合志町の寄主を異にする3個体群、大分県九重町の1個体群、鹿児島県溝辺町の1個体群）を形態観察と計測値の統計的解析により比較した。6個体群の唇部形態は酷似しているが、鹿児島県溝辺産の個体群の唇部前縁の突出は弱く、3体環が頻出する。口針節球の形態の変異には、個体群間で若干の重なりがある。尾端形態では、四街道産の個体群と他の5個体群の間に相違がある。線形判別関数分析により6個体群を3群に判別する際、有意に貢献している形質を探索した。それらは、用いた個体群に関する限り、頭端からの排泄孔の距離、G2(後部子宮枝長/体長の百分率)、口針節球の高さ、V値、m値(口針錐/口針の百分率）の5形質であった。正準判別分析および主成分分析により、これら5形質の有効性が確認された。上記の解析に基づき大分県九重町および熊本県西合志町のイネ科草本に由来する個体群を新種と認め、*Pratylenchus gotohi* と命名・記載した。本種はムギネグサレセンチュウから、上記5形

質の他に尾部体環数（20対17；平均値）および尾端形態の相違で区別できる。本種の種小名は、本種を最初に認識し、国内のネグサレセンチュウ属の種の探索と同定に尽力された後藤昭博士に由来する。原野・放牧地等未耕地から検出されるという生態的特徴を考慮し、本種の和名をマキバネグサレセンチュウとしたい。